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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/621,485	07/16/2003	Mike Mueckler	56029/41936	3332

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EXAMINER	
FERNANDEZ, SUSAN EMILY	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 08/04/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/621,485	MUECKLER ET AL.	
	Examiner	Art Unit	
	Susan E. Fernandez	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 May 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-15 and 22* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-15 and 22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

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DETAILED ACTION

The amendment filed May 20, 2005, has been received and entered.

Claims 1-15 and 22 are pending and are presented for examination.

Specification

The disclosure is objected to because of the following informalities: Page 30 of the specification is missing. It is noted that the applicant has stated that has filed on May 20, 2005 a copy of the originally filed page 30 of the specification. However, page 30 is still not present in the file. Appropriate correction is required.

Claim Objections

Applicant is advised that should claim 5 be found allowable, claim 22 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). In the response filed May 20, 2005, the applicant has stated that the applicant would like to defer addressing the issue until claim 5 is found allowable. However, until the issue is resolved, the objection must be maintained.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-4, 7 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Wijkander et al. (Journal of Biological Chemistry, 1997, 272(34): 21520-21526) in light of Alessi et al. (Current Biology, 1997, 7: 261-269) and Cross et al. (Nature, 1995, 378: 785-789).

Wijkander et al. discloses the homogenization of rat adipocytes in order to obtain supernatants referred to as "cytosol fractions" and pellets referred to as "membrane fractions". See page 21520, last paragraph, through page 21521, first paragraph. The membrane fractions consist of various membranes, including plasma membranes. The cytosol and membrane fractions are present in a homogenization buffer that lacks chloride ions. A protein kinase B assay was performed on these fractions as described on page 21521 under "Protein Kinase Assay", wherein a mixture consisting of ATP and 40 mM $MgCl_2$ is added. Figure 2 provides results obtained from these assays, noting the effect of stimulating cells with insulin prior to obtaining cytosol and membrane fractions. Membrane fractions were also combined with cytosol fractions and a kinase assay was performed of this mixture for both control and stimulated cells (page 21524, second column, last paragraph).

Alessi et al. discloses that "the full activation of PKBα *in vitro* requires the phosphorylation of Ser473 as well as Thr308" (page 266, first sentence).

Cross et al. discloses that Akt (PKB according to the examined application, page 1, third paragraph) phosphorylates GSK3 (page 789, last paragraph).

It is evident that the protein kinase B activated through *in vitro* experimentation in Wijkander et al. would have required the phosphorlyation of a serine and a threonine residue. If the PKB corresponds to SEQ ID NO:1, the phosphorylated residues required for PKB activation are the residues at positions 308 and 473. Furthermore, it is clear that the activated PKB could phosphorylate GSK3. A holding of anticipation is clearly required.

Applicant's arguments filed May 20, 2005 have been fully considered but they are not persuasive. Figure 3 does not demonstrate the results obtained by Wijkander in combining fractions together. Instead, attention should be drawn to page 21524, second column, last paragraph. As pointed out above, a kinase assay had been performed in the Wijkander study, wherein membrane fractions (from control or stimulated cells) were mixed with a cytosol fraction from stimulated cells (page 21524, second column, last paragraph). Kinase activity was detected, though less than what was detected solely in the cytosol fraction (72% inhibition). Nevertheless, the detection of a certain level of kinase activity indicates that protein kinase B was indeed activated.

In response to applicant's arguments against the Alessi and Cross references individually, it is respectfully pointed out that Alessi et al. and Cross et al. are applied in combination with Wijkander et al. Thus, the holding of anticipation is properly maintained.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-4, 7 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al. in view of Alessi et al. and Cross et al.

Wijkander et al. discloses the homogenization of rat adipocytes in order to obtain supernatants referred to as "cytosol fractions" and pellets referred to as "membrane fractions". See page 21520, last paragraph, through page 21521, first paragraph. The membrane fractions consist of various membranes, including plasma membranes. The cytosol and membrane fractions are present in a homogenization buffer that lacks chloride ions. A protein kinase B assay was performed on these fractions as described on page 21521 under "Protein Kinase Assay", wherein a mixture consisting of ATP and 40 mM $MgCl_2$ is added. Figure 2 provides

results obtained from these assays, noting the effect of stimulating cells with insulin prior to obtaining cytosol and membrane fractions. Membrane fractions were also combined with cytosol fractions and a kinase assay was performed of this mixture for both control and stimulated cells (page 21524, second column, last paragraph).

Wijkander et al. does not expressly disclose that the protein kinase B (PKB) is activated by having a phosphorylated threonine residue and a phosphorylated serine residue, or specifically, a phosphorylated threonine residue at position 308 of SEQ ID NO:1 and a phosphorylated serine residue at position 473 of SEQ ID NO:1. Furthermore, Wijkander does not show that the activated protein kinase B is capable of phosphorylating a GSK3.

Alessi et al. discloses that "the full activation of PKBα *in vitro* requires the phosphorylation of Ser473 as well as Thr308" (page 266, first sentence).

Cross et al. discloses that Akt (PKB according to the examined application, page 1, third paragraph) phosphorylates GSK3 (page 789, last paragraph)

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to conclude that the protein kinase B activated through *in vitro* experimentation in Wijkander et al. would have required the phosphorlyation of a serine and a threonine residue. If the PKB corresponds to SEQ ID NO:1, it would have been obvious that the phosphorylated residues required for PKB activation are the residues at positions 308 and 473. Furthermore, it would have been obvious to conclude that the activated PKB could phosphorylate GSK3.

One of ordinary skill in the art would have been motivated to do this because Alessi et al. establishes that phosphorylation of both a serine and a threonine residue is required for *in vitro* activation of PKB. According to the first sentence on page 14 of the application under

examination, PKBα corresponds to the kinase as depicted in SEQ ID NO:1. If the particular PKB to be activated is PKBα, it is evident from Alessi et al. that Ser473 and Thr308 would have been phosphorylated. Additionally, it is clear from Cross et al. that the activated PKB could have the ability to phosphorylate GSK3. A holding of obviousness is clearly required.

Applicant's arguments filed May 20, 2005 have been fully considered but they are not persuasive (see previous section, "*Claim Rejections - 35 USC § 102*"). In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). It is therefore respectfully submitted that the rejection set forth herein is required.

Claims 1-8 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al., Alessi et al., and Cross et al. as applied to claims 1-4, 7 and 8 above, and further in view of Vanhaesebroeck et al. (Biochem. J., 2000, 346: 561-576).

As discussed above, Wijkander et al., Alessi et al., and Cross et al. render claims 1-4, 7 and 8 obvious.

These references do not expressly disclose the addition of PIP3 or PI(3,4)P2 to the membrane and cytosol fraction mixture.

Vanhaesebroeck et al. discloses that the phosphorylation of Thr308 in PKBα is "enhanced over 1000-fold in the presence of lipid vesicles containing low amounts of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2..." (page 565, first column, second paragraph). PtdIns(3,4,5)P_3

and PtdIns(3,4)P_2 correspond to PIP3 and PI(3,4)P2, respectively (page 561). The requirement of including PIP3 and PI(3,4)P2 for enhanced phosphorylation is supported by *in vitro* experimentation (page 565, first column, second paragraph, last sentence).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to add PIP3 and/or PI(3,4)P2 to the combined fraction mixture in order to activate protein kinase B.

One of ordinary skill in the art would have been motivated to add PIP3 and/or PI(3,4)P2 to the reaction mixture because experiments conducted by Wijkander had suggested that the treatment of cells with peroxovandate could result in greater generation of 3-phosphorylated phophoinositides, such as PIP3 and PI(3,4)P2 (page 21524, first column, second paragraph). Figure 6 shows the increased amount of PKB activity detected in the membrane fraction when cells were treated with peroxovandate. Furthermore, Vanhaesebroeck et al. emphasizes that PIP3 and PI(3,4)P2 are "the lipids that are crucial for the activation of PKB" (page 561, second column, second paragraph), thus one of ordinary skill in the art would have been motivated to add PIP3 and PI(3,4)P2 in order to enhance PKB activation. A holding of obviousness is clearly required.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, the rejection set forth herein is maintained.

Claims 1-8 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al., Alessi et al., Cross et al., and Vanhaesebroeck as applied to claims 1-8 and 22 above, and further in view of Bauer et al. (General and Comparative Endocrinology, 1983, 49(3): 414-427).

As discussed above, Wijkander et al., Alessi et al., Cross et al., and Vanhaesebroeck render claims 1-8 and 22 obvious.

These references do not teach the application of their methods to other insulin-responsive cells besides adipocytes.

Bauer et al. discloses that islet cells are insulin-responsive cells (CAPLUS abstract).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to apply the methods used in Wijkander et al. to other insulin-responsive cells, including muscle cells, liver cells, and islet cells.

One of ordinary skill in the art would have been motivated to do this because Wijkander notes that studies are performed with rat adipocytes because there is need for information about insulin regulation of PKB from "insulin-responsive target tissues such as liver, muscle or adipose tissue" (page 21520, second column, second paragraph). One of ordinary skill in the art would therefore have been motivated to try the listed tissues in order to obtain more information about PKB regulation. Experimentation would include islet cells which Bauer indicates are insulin-responsive and resemble other insulin-responsive cells. There is a reasonable expectation of success that the methods applied to adipocytes would translate well when applied to other cells. A holding of obviousness is clearly required.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, the rejection set forth herein is maintained.

Claims 9-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al. in view of Hill et al. (Methods Enzymol., 2002, 345: 448-463), Campbell (Biology, 3rd edition, 1992, Benjamin/Cummings Publishing Co., Inc., page 104), Vanhaesebroeck et al., Alessi et al., and Cross et al.

As discussed above, Wijkander et al. discloses the homogenization of rat adipocytes in order to obtain supernatants referred to as "cytosol fractions" and pellets referred to as "membrane fractions". The membrane fractions consist of various membranes, including plasma membranes. The cytosol and membrane fractions are present in a homogenization buffer that lacks chloride ions. A protein kinase B assay was performed on these fractions as described on page 21521 under "Protein Kinase Assay", wherein a mixture consisting of ATP and 40 mM $MgCl_2$ is added. Figure 2 provides results obtained from these assays, noting the effect of stimulating cells with insulin prior to obtaining cytosol and membrane fractions. Membrane fractions were also combined with cytosol fractions and a kinase assay was performed of this mixture for both control and stimulated cells.

Wijkander et al. does not expressly disclose treating the membrane fraction (which contains plasma membrane) with a high salt solution, thus obtaining a salt-extracted plasma

membrane fraction and an aqueous fraction which is desalted. Furthermore, it does not disclose combining the salt-extracted plasma membrane fraction with the desalted aqueous fraction, the cytoplasmic fraction, ATP, and a phosphatidylinositol phosphate molecule in a buffer comprising less than 145 mM chloride. Additionally, Wijkander et al. does not expressly disclose that the protein kinase B (PKB) is activated by having a phosphorylated threonine residue and a phosphorylated serine residue, or specifically, a phosphorylated threonine residue at position 308 of SEQ ID NO:1 and a phosphorylated serine residue at position 473 of SEQ ID NO:1. Finally, Wijkander does not show that the activated protein kinase B is capable of phosphorylating a GSK3.

Hill et al. discloses that "membrane translocation is an important event in the stimulation of PKB activity, thus, in addition to monitoring the phosphorylation status and kinase activity, it is also desirable to determine the subcellular localization of PKB in response to different stimuli" (page 458, last paragraph through page 459, first paragraph). Furthermore, Hill et al. states that "preparation of subcellular fractions enriched in the plasma membrane allows the comparison of the relative proportion of membrane-bound and cytosolic PKB before and after stimulation" (page 460, second paragraph). Preparation of a crude plasma membrane fraction is outlined on pages 461 and 462, wherein buffers used comprise less than 145 mM chloride. The paragraph under "Analysis of Subcellular Fractions" on page 462 indicates that the crude plasma membrane pellet is mixed with NP-40 lysis buffer (page 452) which comprises 50 mM Tris-HCl and 120 mM NaCl in order to remove insoluble material. Thus the crude plasma membrane pellet is in a solution comprising 170 mM chloride.

Campbell discloses that enzymes are "sensitive to salt concentration" and that "most enzymes cannot tolerate extremely saline (salty) solutions" (page 104, last paragraph).

Vanhaesebroeck et al. discloses that the phosphorylation of Thr308 in PKBα is "enhanced over 1000-fold in the presence of lipid vesicles containing low amounts of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2..." (page 565, first column, second paragraph). PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 correspond to PIP3 and PI(3,4)P2, respectively (page 561). The requirement of including PIP3 and PI(3,4)P2 for enhanced phosphorylation is supported by *in vitro* experimentation (page 565, first column, second paragraph, last sentence).

Alessi et al. discloses that "the full activation of PKBα *in vitro* requires the phosphorylation of Ser473 as well as Thr308" (page 266, first sentence).

Cross et al. discloses that Akt (PKB according to the examined application, page 1, third paragraph) phosphorylates GSK3 (page 789, last paragraph).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to mix the membrane fraction obtained in Wijkander et al. with the NP-40 lysis buffer as described in Hill et al. Furthermore, since this buffer would extract the plasma membrane, it would have been obvious to a person of ordinary skill in the art to desalt the aqueous fraction obtained. It would have been obvious to combine the resulting fractions with all other ingredients described in Wijkander et al. It would have been obvious to add a phosphatidylinositol phosphate, such as PIP3 and/or PI(3,4)P2, to the combined fraction mixture in order to activate protein kinase B. Additionally, it would have been obvious to conclude that the protein kinase B activated through *in vitro* experimentation in Wijkander et al. would have required the phosphorlyation of a serine and a threonine residue. If the PKB corresponds to SEQ

ID NO:1, it would have been obvious that the phosphorylated residues required for PKB activation are the residues at positions 308 and 473. Furthermore, it would have been obvious to conclude that the activated PKB could phosphorylate GSK3.

One of ordinary skill in the art would have been motivated to solubilize the membrane fraction in the high salt NP-40 lysis buffer in order to remove insoluble material that may interfere with kinase activity or protein concentration assays. The aqueous fraction obtained through the salt-extraction would have required desalting for its use in a reaction mixture because Campbell shows that protein activity can be detrimentally affected by salt solution concentration. It would have been obvious to add a phosphatidylinositol phosphate, such as PIP3 and PI(3,4)P2, because Vanhaesebroeck et al. emphasizes that PIP3 and PI(3,4)P2 are "the lipids that are crucial for the activation of PKB". Thus one of ordinary skill in the art would have been motivated to add PIP3 and PI(3,4)P2 in order to enhance PKB activation. Alessi et al. establishes that phosphorylation of both a serine and a threonine residue is required for *in vitro* activation of PKB, thus it would have been obvious that this would have been required in order to practice the Wijkander experiments. According to the first sentence on page 14 of the application under examination, PKBα corresponds to the kinase as depicted in SEQ ID NO:1. If the particular PKB to be activated is PKBα, it is evident from Alessi et al. that Ser473 and Thr308 would have been phosphorylated. Additionally, it is clear from Cross et al. that the activated PKB could have the ability to phosphorylate GSK3. A holding of obviousness is clearly required.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on

combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, the rejection set forth herein is maintained.

Claims 9-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al., Hill et al., Campbell, Vanhaesebroeck et al., Alessi et al. and Cross et al. as applied to claims 9-15 above, and further in view of Bauer et al.

As discussed above, Wijkander et al., Hill et al., Campbell, Vanhaesebroeck et al., Alessi et al. and Cross et al. render claims 9-15 obvious.

These references do not teach the application of their methods to other insulin-responsive cells besides adipocytes.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to apply the methods used in Wijkander et al. to other insulin-responsive cells, including muscle cells, liver cells, and islet cells.

One of ordinary skill in the art would have been motivated to do this because Wijkander notes that studies are performed with rat adipocytes because there is need for information about insulin regulation of PKB from "insulin-responsive target tissues such as liver, muscle or adipose tissue" (page 21520, second column, second paragraph). One of ordinary skill in the art would therefore have been motivated to try the listed tissues in order to obtain more information about PKB regulation. Experimentation would include islet cells which Bauer indicates are insulin-responsive and resemble other insulin-responsive cells. There is a reasonable expectation of

success that the methods applied to adipocytes would translate well when applied to other cells. A holding of obviousness is clearly required.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, the rejection set forth herein is maintained.

Claims 9-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al. in view of Hill et al. (Current Biology, 2002, 12(14): 1251-1255), Campbell, Vanhaesebroeck et al., Alessi et al., and Cross et al.

As discussed above, Wijkander et al. discloses the homogenization of rat adipocytes in order to obtain supernatants referred to as "cytosol fractions" and pellets referred to as "membrane fractions". The membrane fractions consist of various membranes, including plasma membranes. The cytosol and membrane fractions are present in a homogenization buffer that lacks chloride ions. A protein kinase B assay was performed on these fractions as described on page 21521 under "Protein Kinase Assay", wherein a mixture consisting of ATP and 40 mM $MgCl_2$ is added. Figure 2 provides results obtained from these assays, noting the effect of stimulating cells with insulin prior to obtaining cytosol and membrane fractions. Membrane fractions were also combined with cytosol fractions and a kinase assay was performed of this mixture for both control and stimulated cells.

Wijkander et al. does not expressly disclose treating the membrane fraction (which containins plasma membrane) with a high salt solution, thus obtaining a salt-extracted plasma membrane fraction and an aqueous fraction which is desalted. Furthermore, it does not disclose combining the salt-extracted plasma membrane fraction with the desalted aqueous fraction, the cytoplasmic fraction, ATP, and a phosphatidylinositol phosphate molecule in a buffer comprising less than 145 mM chloride. Additionally, Wijkander et al. does not expressly disclose that the protein kinase B (PKB) is activated by having a phosphorylated threonine residue and a phosphorylated serine residue, or specifically, a phosphorylated threonine residue at position 308 of SEQ ID NO:1 and a phosphorylated serine residue at position 473 of SEQ ID NO:1. Finally, Wijkander does not show that the activated protein kinase B is capable of phosphorylating a GSK3.

Hill et al. (Current Biology) discloses the use of a high salt solution (0.5 M NaCl) in order to extract the plasma membrane fraction obtained from human embryonic kidney (HEK) 293 cells. Analysis of the salt-extracted plasma membrane fraction allowed for isolation of a "constitutively active, membrane lipid raft-associated kinase activity that phosphorylates PKB on Ser473 and is distinct from ILK, PDK1, and PKB" (page 1254, last paragraph through page 1255, first paragraph).

Campbell discloses that enzymes are "sensitive to salt concentration" and that "most enzymes cannot tolerate extremely saline (salty) solutions" (page 104, last paragraph).

Vanhaesebroeck et al. discloses that the phosphorylation of Thr308 in PKBα is "enhanced over 1000-fold in the presence of lipid vesicles containing low amounts of PtdIns(3,4,5)P_3 or PtdIns(3,4)P_2..." (page 565, first column, second paragraph). PtdIns(3,4,5)P_3

and PtdIns(3,4)P_2 correspond to PIP3 and PI(3,4)P2, respectively (page 561). The requirement of including PIP3 and PI(3,4)P2 for enhanced phosphorylation is supported by *in vitro* experimentation (page 565, first column, second paragraph, last sentence).

Alessi et al. discloses that "the full activation of PKBα *in vitro* requires the phosphorylation of Ser473 as well as Thr308" (page 266, first sentence).

Cross et al. discloses that Akt (PKB according to the examined application, page 1, third paragraph) phosphorylates GSK3 (page 789, last paragraph).

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to obtain a salt-extracted plasma membrane fraction which would have been combined with the other fraction(s) and ingredients for PKB activation. It would have been obvious to a person of ordinary skill in the art to desalt the aqueous fraction obtained and include the aqueous fraction in the reaction mixture. It would have been obvious to a person of ordinary skill in the art to add a phosphatidylinositol phosphate, such as PIP3 and/or PI(3,4)P2, to the combined fraction mixture in order to activate protein kinase B. Additionally, it would have been obvious to conclude that the protein kinase B activated through *in vitro* experimentation in Wijkander et al. would have required the phosphorlyation of a serine and a threonine residue. If the PKB corresponds to SEQ ID NO:1, it would have been obvious that the phosphorylated residues required for PKB activation are the residues at positions 308 and 473. Furthermore, it would have been obvious to conclude that the activated PKB could phosphorylate GSK3.

One of ordinary skill in the art would have been motivated to obtain a salt-extracted plasma membrane fraction because Hill et al. (Current Biology) states that it allows for the isolation of active kinase(s) that phosphorylate Ser473 in PKBα, which is shown by Alessi et al

as necessary for *in vitro* PKBα activation. The aqueous fraction obtained through the salt-extraction would have required desalting for its use in a reaction mixture because Campbell shows that protein activity can be detrimentally affected by salt solution concentration. Combination of all fractions would have ensured that all required proteins for optimal *in vitro* PKB activation are included. It would have been obvious to add a phosphatidylinositol phosphate, such as PIP3 and PI(3,4)P2, because Vanhaesebroeck et al. emphasizes that PIP3 and PI(3,4)P2 are "the lipids that are crucial for the activation of PKB". Thus one of ordinary skill in the art would have been motivated to add PIP3 and PI(3,4)P2 in order to enhance PKB activation. One of ordinary skill in the art would have arrived at the above conclusions because Alessi et al. establishes that phosphorylation of both a serine and a threonine residue is required for *in vitro* activation of PKB. According to the first sentence on page 14 of the application under examination, PKBα corresponds to the kinase as depicted in SEQ ID NO:1. If the particular PKB to be activated is PKBα, it is evident from Alessi et al. that Ser473 and Thr308 would have been phosphorylated. Additionally, it is clear from Cross et al. that the activated PKB could have the ability to phosphorylate GSK3. A holding of obviousness is clearly required.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, the rejection set forth herein is maintained.

Claims 9-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wijkander et al., Hill et al. (Current Biology), Campbell, Vanhaesebroeck et al., Alessi et al. and Cross et al. as applied to claims 9-15 above, and further in view of Bauer et al.

As discussed above, Wijkander et al., Hill et al. (Current Biology), Campbell, Vanhaesebroeck et al., Alessi et al. and Cross et al. render claims 9-15 obvious.

These references do not teach the application of their methods to other insulin-responsive cells besides adipocytes.

At the time the invention was made, it would have been obvious to a person of ordinary skill in the art to apply the methods used in Wijkander et al. to other insulin-responsive cells, including muscle cells, liver cells, and islet cells.

One of ordinary skill in the art would have been motivated to do this because Wijkander notes that studies are performed with rat adipocytes because there is need for information about insulin regulation of PKB from "insulin-responsive target tissues such as liver, muscle or adipose tissue" (page 21520, second column, second paragraph). One of ordinary skill in the art would therefore have been motivated to try the listed tissues in order to obtain more information about PKB regulation. Experimentation would include islet cells which Bauer indicates are insulin-responsive and resemble other insulin-responsive cells. There is a reasonable expectation of success that the methods applied to adipocytes would translate well when applied to other cells. A holding of obviousness is clearly required.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re*

Merck & Co., 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Thus, the rejection set forth herein is maintained.

No claims are allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan E. Fernandez whose telephone number is (571) 272-3444. The examiner can normally be reached on Mon-Fri 8:30 am - 5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on (571) 272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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PRIMARY EXAMINER